PSEUDOMONAS AERUGINOSA IN BOVINE MASTITIS [1]

By J. FRANK CONE

Associate market-milk specialist, Division of Market-Milk Investigations, Bureau of Dairy Industry, United States Department of Agriculture [2]

INTRODUCTION

Small, Gram-negative bacteria that produced a blue-green fluorescence characteristic of *Pseudomonas aeruginosa* (*Bacillus pyocyaneus*) were found in all but 2 cases of an unusually severe outbreak of bovine mastitis involving 23 cows. Since the outbreak was unusual in character, a study was carried on to determine the identity of the organisms and their possible significance. The results are reported in this paper.

REVIEW OF LITERATURE

The occurrence of *Pseudomonas aeruginosa* in the mammary glands has seldom been reported. In fact, Carpenter (*3*) [3] states that he attempted artificial inoculation of a normal udder with *Bacterium pyocyaneum* without causing more than temporary mild symptoms of mastitis. Natural invasion of the udder was indicated when Mundhenk (*13*) found *Bacillus pyocyaneus* in milk samples from cows with severe cases of mastitis, but he considered the organisms only secondary invaders associated with colon bacilli.

Pickens, Welch, and Poelma (*15*) studied an outbreak of mastitis that they attributed to *Pseudomonas aeruginosa*, in which agglutination tests with *Ps. aeruginosa* antigen on blood from cows with infected udders showed titers as high as 1:1,000 in some cases, whereas the blood from cows with normal udders showed titers of 1:50 or less. Some of the cows had periodic relapses, but gave apparently normal milk between attacks. Cherrington and Gildow (*4*) also reported recurring attacks of acute mastitis in which *Ps. aeruginosa* was found in the milk in almost pure culture. Their findings were likewise supported with agglutination tests. They attributed the recurrent attacks to a water supply contaminated with *Ps. aeruginosa*.

NATURE OF THE OUTBREAK

The outbreak reported herein occurred in the Bureau of Dairy Industry herd at Beltsville, Md., where samples of milk were being collected periodically for examination for indications of mastitis. Small, Gram-negative rods giving the characteristic green fluorescence of *Pseudomonas aeruginosa* were first isolated on March 29, 1937 from milk from the left front quarter of cow 1247. An abrupt rise in

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[3] Italic numbers in parentheses refer to Literature Cited, p. 00.

chloride content and leucocyte count of the milk from the infected quarter indicated a mild case of mastitis.

On April 24 cow 880 suffered a sudden attack of mastitis. Her milk production dropped from a normal of 65 pounds on the previous day to 30 pounds, but gradually returned to almost normal in 4 days. A milk sample from the left rear quarter taken April 27 yielded many green fluorescent, Gram-negative rods. On May 3 the organisms were isolated from a third cow that showed mild symptoms of mastitis.

On May 10 the disease broke out in epizootic proportions. Between then and June 11 milk samples from the infected quarters of 17 cows suffering from severe mastitis were found to contain the green fluorescent rods in apparently pure culture. Many of the affected animals were obviously sick, one animal running a temperature as high as 106.8° F. Milk production dropped abruptly, in some cases to almost nothing. The milk from the affected quarters was watery, yellow, and very lumpy and stringy. The number of pus cells, as obtained in a direct smear, were estimated to be more than 50,000,000 per milliliter. The records of cows A–116 and A–118 (table 1) well illustrate the course of the disease.

TABLE 1.—*Milk-production records of cows A–116 and A–118*

Date	Milk production of Cow A–116 [1]			
	Morning milking	Noon milking	Evening milking	Total
	Pounds	*Pounds*	*Pounds*	*Pounds*
May 14	----------	----------	----------	30.1
May 15	----------	----------	----------	30.2
May 16	11.0	11.9	8.3	31.2
May 17	11.5	7.0	5.4	23.9
May 18	4.7	4.4	2.7	11.8
May 19	6.9	4.6	1.3	12.8
May 20	5.8	6.7	4.1	16.6
May 21	7.4	7.8	5.0	20.2
May 22	8.9	8.5	6.3	23.7
May 23	10.0	8.0	7.2	25.2
May 30	10.6	10.4	7.7	28.7

Date	Milk production of cow A–118 [2]			
	Morning milking	Noon milking	Evening milking	Total
	Pounds	*Pounds*	*Pounds*	*Pounds*
May 29	14.2	14.3	13.4	41.9
May 30	15.6	16.3	11.1	43.0
May 31	11.2	16.0	3.7	30.9
June 1	4.5	3.6	0.4	8.5
June 2	2.0	0.2	0.4	2.6
June 3	1.5	1.6	0.5	3.6
June 4	2.8	3.7	2.4	8.9
June 5	5.8	5.3	4.0	15.1
June 8	14.0	6.4	4.7	25.1
June 15	12.1	9.2	6.0	27.3

[1] Rectal temperature: May 18, 106° F.; May 24, 103°; May 25, 102.4°. Bacteriological examination: Green fluorescent rods were found in the milk for May 18, 22, and June 4; none was found in milk of June 9.

[2] Rectal temperature: May 31, 106.7° F.; June 4, 103°. Bacteriological examination: Green fluorescent rods were found in the milk May 31, June 1, 4, 9, 11, and 18; none was found in milk of Aug. 16 and Dec. 13. A year after the onset of the disease the quarter was still hard and swollen, and the milk was alkaline to bromocresol purple.

As a result of the infection one cow lost a quarter permanently and another cow lost one temporarily. With some of the other cows the affected quarters have remained more or less hard and swollen, although producing milk of apparently normal appearance. For most of the cows few data are available regarding the persistence with which the green fluorescent rods were shed into the milk after the acute attack had cleared up. Only two of the infected cows (1441 and 1445) remained on regular semimonthly sampling. When cow 1441 freshened on December 13, all four quarters were producing. At that time and again on February 28, 1938, she had recurrences of mild mastitis, with large numbers of the organisms present in the milk. Cow 1445 continued shedding the organisms into the milk until she went dry in October. After freshening again on January 9, 1938, she continued to suffer mild recurrences of mastitis in the affected quarter and the organisms were found in the milk on January 24, February 28, and May 23. The milk from that quarter has continued to be higher in chloride content and leucocyte count and slightly more alkaline to bromocresol purple than the milk from her normal quarters.

Only one new case of mastitis was found after June 11, 1937. A sample of milk taken August 31 from the right rear quarter of cow 1236 showed a few of the green fluorescent rods and a slight elevation of the chloride content and the leucocyte count. She showed no further disturbances from then until she was removed from the herd in December.

CHARACTERISTICS OF THE ORGANISMS ISOLATED

A pure-culture study of 18 strains of the green fluorescent organisms isolated from the infected quarters showed that their characteristics agree quite closely with those described in Bergey's Manual (*1*) for *Pseudomonas aeruginosa* except in two important features—their failure to produce indole and their ability to produce acid from certain carbohydrates. Bergey considered indole production sufficiently constant to justify the use of this characteristic in his key to separate *Ps. aeruginosa* from those species not producing indole.

A search of the literature on *Pseudomonas aeruginosa*, however, revealed an extreme lack of agreement on both indole production and sugar fermentations. Chester (*5*) described the species as indole-negative. Pickens and coworkers (*15*), Cherrington and Gildow (*4*), Buonomini (*2*), Fuller and Johnson (*7*), and Stark and Scheib (*20*) found no indole production. Jordon (*10*) found three out of seven strains producing indole and four not producing it. Lartigau (*11*) and Růžička (*16*) found that indole was produced. Sherwood, Johnson, and Radotincky (*18*) studied 22 strains obtained from various laboratories and found that all produced indole with Nelson's (*14*) vanillin test.

In addition to the 18 strains isolated from milk produced by cows affected with mastitis, 4 strains of *Pseudomonas aeruginosa* obtained from other laboratories (including No. 256 and No. 914 from the American type culture collection) were tested for indole production. The organisms were grown for 72 hours at 37° C. in Bacto-tryptophane broth. Determinations for indole were made by the Goré test and the Ehrlich-Böhme test described in the Manual of Methods for Pure Culture Study (*19*) and by the vanillin test of Nelson (*14*). None of

the strains tested gave a positive reaction by any of the three methods.

The reason for the conflicting reports regarding the ability of *Pseudomonas aeruginosa* to ferment carbohydrates is more understandable than in the case of indole production. Sears and Gourley (*17*) have shown that certain carbohydrates are utilized by *Ps. aeruginosa*, but that the production of an acid reaction depends upor the nitrogen content of the medium. They found that in a glucose broth containing 1 percent or less of peptone the glucose was attacked and the reaction dropped to between pH 5.0 and 4.0. When higher concentrations of peptone were employed, the production of ammonia from the break-down of the peptone was sufficiently rapid to neutralize the acid produced from the glucose and keep the reaction of the medium neutral or alkaline. Liot (*12*), Goris and Liot (*8*), and Supniewski (*21*) also have shown that acids produced by *Bacillus pyocyaneus* from the fermentation of carbohydrates may be neutralized by the ammonia produced.

In the studies by the author bromothymol blue was chosen as the indicator for use in the fermentation tests because it shows slight changes in reaction either way from neutrality. The basic medium was beef-extract broth at pH 7.0, to which was added 1 ml of a 1.6-percent alcoholic solution of bromothymol blue per liter. This medium was tubed and sterilized, after which sterile carbohydrate solutions were added aseptically. The concentrations were so adjusted that the final broth contained 0.3 percent of beef extract, 0.5 percent of peptone, and 1.0 percent of carbohydrate. After the addition of the carbohydrate solutions all tubes were tested for sterility by incubation at 32° C. for 24 hours. Each of these tubes was then inoculated with 1 drop of 24-hour-old broth culture of the test organism grown at 37°. For comparison each culture was also inoculated into a tube of broth containing no carbohydrate, and uninoculated controls of each carbohydrate medium were incubated with the culture tubes. Incubation was at 37°. Readings were taken after 1, 3, and 6 days of incubation. The 18 strains obtained from mastitis milk and 4 strains of *Pseudomonas aeruginosa* obtained from other laboratories were tested.

All of the strains were identical in their fermentation reactions. Decided or slight acid reactions were obtained in media containing arabinose, xylose, dextrose, levulose, galactose, glycerol, and mannitol. Very alkaline reactions were obtained in the medium containing no carbohydrate and in the media containing sucrose, maltose, lactose, raffinose, inulin, dextrin, and dulcitol. It may be significant that all the carbohydrates that apparently were fermented contained six carbon atoms or less and all that were not fermented, except dulcitol, contained more than six carbon atoms.

Splitting of fat tested for by the method described by Hammer and Collins (*9*), and other biochemical tests made by methods described in the Manual of Methods for Pure Culture Study (*19*), showed further characteristics common to all the strains to be: Gelatin was liquefied; milk was coagulated and proteolyzed; nitrates were reduced rapidly to nitrites and then to free nitrogen; butterfat was split; starch was not hydrolyzed; and hydrogen sulphide was not formed. All the strains from mastitis milk were motile by means of single polar flagellae. When first isolated all strains produced a chloroform-soluble blue

pigment characteristic of pyocyanin. This characteristic was lost by some strains on prolonged laboratory cultivation.

As previously stated, the ability of *Pseudomonas aeruginosa* to ferment carbohydrates and to produce indole are questions upon which various workers have failed to agree. But since all the other cultural and morphological characteristics determined for the strains in these studies, especially the ability of the freshly isolated cultures to produce pyocyanin, agree with those generally recognized as typical for *Ps. aeruginosa*, it was decided that the strains under study are members of the species *Ps. aeruginosa*.

DISCUSSION

Frobisher (*6*) states that *Pseudomonas aeruginosa*—

> probably has not the power of invading healthy tissues but sometimes gains a foothold and grows in already diseased loci, living a saprophytic existence there, possibly producing substances which result in the death and destruction of already injured cells.

That such was the case in the outbreak reported herein seems unlikely. The herd was under close observation for mastitis prior to the outbreak, and it is significant that although some streptococcic mastitis existed in the herd, most of the individuals involved in this study had had no previous history of mastitis. Two cases of acute mastitis from which coliform organisms were found in apparently pure culture occurred in the herd during the outbreak reported in this paper. In no instance was a mixed infection found and in no instance were other organisms which appeared to be significant found in any quarter either immediately before or immediately after the finding of *Ps. aeruginosa*. Although agglutination tests were inconclusive, it is concluded that *Ps. aeruginosa* was the sole etiological agent in the cases in which it was found.

From the standpoint of public health the significance of the presence of *Pseudomonas aeruginosa* in the udder is problematical; nevertheless it should not be dismissed too lightly. There are numerous references in the literature to cases of enteritis in which *Ps. aeruginosa* was considered the causative agent. Lartigau (*11*) reported outbreaks of dysentery attributed to *Bacillus pyocyaneus* in two families in which there was a total of 15 cases and 4 deaths. The source of infection in each outbreak was a contaminated well from which the family used water. In the present case the organisms were shed persistently into the milk long after the milk secretion had become apparently normal in appearance, and would be a constant source of infection to those who might be drinking raw milk from the infected animals.

The extremely active proteolysis and lipolysis exhibited by *Pseudomonas aeruginosa* makes it a factor to contend with also in the deterioration of stored dairy products. North [4] has frequently found *Ps. aeruginosa* in cream, and has observed that when initially present in relatively high numbers this organism caused spoilage at temperatures from 7° to 30° C. Stark and Scheib (*20*) found that *Ps. aeruginosa*

[4] NORTH, W. R. Personal communication. 1938.

was frequently the cause of butter deterioration. Speaking of the Gram-negative group as a whole, they state (*20, pp. 209–210*):

Since 97 per cent (154 cultures) of these organisms hydrolyzed fat, and all of them split tributyrin, it would seem that the presence of casein-digesting gram negative rods in butter or cream might well be used as an indication of the probable spoilage of butter. Small numbers of these proteolytic gram negative rods found in fresh lightly salted, sweet cream butter, held at temperatures which would permit the growth of these organisms, was invariably followed by large numbers of the organisms and a poor quality butter.

Forty of the 154 cultures they considered in this group were *Ps. aeruginosa*.

SUMMARY

Pseudomonas aeruginosa was found to be associated with and probably the cause of an outbreak of mastitis of extreme severity. The onset was sudden and was accompanied by fever. With some cows milk production practically ceased during the acute stage of the disease. The affected quarters became blind in two cases and in others they remained hard and swollen, although secreting milk of normal appearance.

Some of the cows continued shedding the organisms in small numbers long after the acute attack had subsided and the milk had become normal in appearance.

The strains of *Pseudomonas aeruginosa* isolated from mastitis cases and four strains obtained from other laboratories differed from Bergey's description of the species in their inability to produce indole and their ability to ferment the simpler carbohydrates.

The public-health and economic significance of *Pseudomonas aeruginosa* in milk is discussed briefly.

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